U.S.S.N. 10/018,761 Response filed 17 August 2004 Restriction issued 26 July 2004



Amendments to the Claims:

Please replace the pending claim with the amended claim having the same claim number. The listing of claim will replace all prior versions and listings of claims in the application:

- 1. (currently amended) A method of inhibiting atrophy in mammalian skeletal muscle cells comprising treating the cells with an inhibitor of the Ras/Raf/Mek/Erk pathway.
- 2. (currently amended) The method of [Claim] claim 1, wherein the inhibitor inhibits Ras.
- 3. (withdrawn) The method of Claim 1 wherein the inhibitor inhibits Raf.
- 4. (withdrawn) The method of Claim 1 wherein the inhibitor inhibits Mek.
- 5. (withdrawn) The method of Claim 1 wherein the inhibitor inhibits Erk.
- 6. (currently amended) The method of [Claim] <u>claim</u> 1, wherein the inhibitor is PD98059 or farnesyl transferase.
- 7. (withdrawn) A method of identifying an agent that inhibits atrophy in skeletal muscle cells comprising:
 - (a) preparing muscle cells that express constitutively active mutant forms Ras/Raf/Mek/Erk;
 - (b) subjecting the cells to a test agent;
 - (c) measuring the amount of atrophy in the muscle cells subjected to a test agent;
- (d) comparing the amount of atrophy in the muscle cells subjected to a test agent with the amount of atrophy in untreated transgenic muscle cells of step (a), wherein a smaller amount of atrophy in the muscle cells subjected to a test agent indicates that the agent inhibits the Ras/Raf/Mek/Erk pathway and therefore inhibits atrophy in muscle cells.
- 8. (withdrawn) The method of Claim 7 wherein the measuring utilizes muscle cell diameter, protein amount, p70S6 kinase activation or Phas-1 activation.
- 9. (withdrawn) The method of Claim 7 wherein the measuring utilizes measuring inhibition of Ras/Raf/Mek/Erk.
- 10. (withdrawn) The method of Claim 7 wherein the muscle cells are cultured cells.

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- 11. (withdrawn) The method of Claim 10 wherein the cultured cells are myoblasts.
- 12. (withdrawn) The method of Claim 11 wherein the myoblasts are C2C12 cells.
- 13. (withdrawn) The method of Claim 11 wherein the myoblasts are differentiated myoblasts.
- 14. (withdrawn) The method of Claim 13 wherein the differentiated myoblasts are myotubes.
- 15. (withdrawn) The method of Claim 7 wherein the muscle cells are obtained from a transgenic organism.
- 16. (withdrawn) The method of Claim 7 wherein the muscle cells are within a transgenic organism.
- 17. (withdrawn) The method of Claim 15 wherein the transgenic organism is a transgenic fly, worm, bird, chicken, turkey, mouse, rat, dog, cat, rabbit, sheep, pig, goat or horse.
- 18. (withdrawn) The method of Claim 16 wherein the transgenic organism is a transgenic transgenic fly, worm, bird, chicken, turkey, mouse, rat, dog, cat, rabbit, sheep, pig, goat or horse.
- 19. (withdrawn) A method of identifying an agent that inhibits atrophy in muscle cells comprising:
 - a) measuring the activation of the Ras/Raf/Mek/Erk pathway in untreated muscle cells,
 - b) subjecting the muscle cells that express the Ras/Raf/Mek/Erk pathway to a test agent,
- c) measuring the amount of Ras/Raf/Mek/Erk activity in the muscle cells subjected to a test agent;
- d) comparing the amount of Ras/Raf/Mek/Erk activity in the muscle cells subjected to a test agent with the amount in the untreated muscle cells, whereby a larger amount in the muscle cells treated with a test agent indicates that the agent inhibits the Ras/Raf/Mek/Erk pathway and therefore istinhibits atrophy in muscle cells.
- 20. (withdrawn) A method of identifying a gene encoding a gene product that inhibits skeletal muscle atrophy comprising:
 - (a) preparing muscle cells that express constitutively active mutant forms Ras/Raf/Mek/Erk;
- (b) introducing into the cells of (a) a test gene under conditions in which the test gene encodes a product;
 - (c) measuring the amount of atrophy in the test-gene encoding muscle cells; and

- (d) comparing the amount of atrophy in the test-gene encoding cells with the amount of atrophy in the muscle cells of step (a) in which the test gene has not been introduced, wherein a smaller amount of atrophy in the test gene-encoding muscle cells indicates that the test gene product inhibits the Ras/Raf/Mek/Erk pathway and therefore inhibits atrophy in muscle cells.
- 21. (withdrawn) The method of Claim 20 wherein the measuring utilizes muscle cell diameter, protein amount, p70S6 kinase activation or Phas-1 activation.
- 22. (withdrawn) The method of Claim 20 wherein the muscle cells are cultured cells.
- 23. (withdrawn) The method of Claim 22 wherein the cultured cells are myoblasts.
- 24. (withdrawn) The method of Claim 23 wherein the myoblasts are differentiated myoblasts.
- 25. (withdrawn) The method of Claim 20 wherein the muscle cells are obtained from a transgenic organism.
- 26. (withdrawn) The method of Claim 20 wherein the muscle cells are within a transgenic organism.
- 27. (withdrawn) The method of Claim 25 wherein the transgenic organism is a transgenic fly, worm, bird, chicken, turkey, mouse, rat, dog, cat, rabbit, sheep, pig, goat or horse.
- 28. (withdrawn) The method of Claim 26 wherein the transgenic organism is a transgenic fly, worm, bird, chicken, turkey, mouse, rat, dog, cat, rabbit, sheep, pig, goat or horse.
- 29. (withdrawn) A method of inhibiting atrophy in a vertebrate animal having an atrophy-inducing condition comprising treating the vertebrate animal with an effective amount of an inhibitor of Ras, Raf, Mek or Erk.
- 30. (withdrawn) The method of Claim 29 wherein the vertebrate animal is a chicken, rodent, rabbit, dog, cat, cow, horse, pig, sheep, primate or human.
- 31. (withdrawn) The method of Claim 29 wherein the vertebrate animal is treated prior to exposure to or onset of the atrophy-inducing condition.
- 32. (withdrawn) The method of Claim 29 wherein the atrophy-inducing condition is immobilization.

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- 33. (withdrawn) The method of Claim 29 wherein the atrophy-inducing condition is denervation, starvation, nutritional deficiency, metabolic stress, diabetes, aging, muscular dystrophy or myopathy.
- 34. (currently amended) A method of causing muscle hypertrophy in mammalian skeletal muscle cells, comprising treating the cells with an inhibitor of the Ras/Raf/Mek/Erk pathway.
- 35. (currently amended) The method of [Claim] claim 34, wherein the inhibitor inhibits Ras.
- 36. (withdrawn) The method of Claim 34 wherein the inhibitor inhibits Raf.
- 37. (withdrawn) The method of Claim 34 wherein the inhibitor inhibits Mek.
- 38. (withdrawn) The method of Claim 34 wherein the inhibitor inhibits Erk.
- 39. (currently amended) The method of [Claim] <u>claim</u> 34, wherein the inhibitor is PD98059 or farnesyl transferase.
- 40. (withdrawn) The method of Claim 34 wherein the muscle cells are within a vertebrate animal.
- 41. (withdrawn) The method of Claim 40 wherein the vertebrate animal is a chicken, rodent, rabbit, dog, cat, cow, horse, pig, sheep, primate or human.